

Serial No.: 09/944,448
Filed: August 30, 2001

LISTING OF CLAIMS

This listing of claims will replace all prior versions of claims in the application:

1. (currently amended) A process for making method for establishing undifferentiated human, comprising the steps of:
 - (a) thawing a cryopreserved human blastocyst embryo; and
 - (b) removing trophectoderm from said embryo using anti-human lymphocyte antibody to isolate inner cell mass; and
 - (c) culturing at least a portion of said human blastocyst embryo inner cell mass on a medium capable of sustaining undifferentiated embryonic stem cells, whereby undifferentiated human embryonic stem cells are established.
2. (canceled)
3. (currently amended) The process method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said human blastocyst embryo comprises a human embryo that was cryopreserved from about 5 days to about 6 days after fertilization of said embryo.
4. (currently amended) The process method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said human blastocyst embryo has been cryogenically stored for more than four years.
5. (currently amended) The process method for establishing undifferentiated human embryonic stem cells of claim 1, wherein said thawing step comprises:
 - (a) a first step of treating said cryopreserved human blastocyst embryo with a first solution comprising human follicular fluid and cryoprotectant;
 - (b) a subsequent second step of treating said cryopreserved human blastocyst embryo with a second solution comprising human follicular fluid and cryoprotectant; wherein said second solution comprises a decreased concentration of cryoprotectant relative to said first solution.
6. (currently amended) The process method for establishing undifferentiated human embryonic stem cells of claim 5, wherein said cryoprotectant is selected from the group consisting of sucrose, glycerol and a combination of sucrose and glycerol.

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7. (currently amended) The ~~process method for establishing undifferentiated human embryonic stem cells~~ of claim 1, wherein said thawing step consists of:

- (a) a first step of treating said cryopreserved human blastocyst embryo with a first solution comprising human follicular fluid and cryoprotectant;
- (b) a subsequent second step of treating said cryopreserved human blastocyst embryo with a second solution comprising human follicular fluid and cryoprotectant;
- (c) a subsequent third step of treating said cryopreserved human blastocyst embryo with a third solution comprising hFF and cryoprotectant;
- (d) a subsequent fourth step of treating said cryopreserved human blastocyst embryo with a fourth solution comprising hFF and cryoprotectant; wherein said fourth solution comprises a decreased concentration of cryoprotectant relative to said third solution, said third solution comprises a decreased concentration of cryoprotectant relative to said second solution, and said second solution comprises a decreased concentration of cryoprotectant relative to said first solution.

8. (currently amended) The ~~process method for establishing undifferentiated human embryonic stem cells~~ of claim 5, further comprising a subsequent third step of treating said cryopreserved human blastocyst embryo with a third solution comprising hFF and cryoprotectant; wherein said third solution comprises about 0.1-2 vol % glycerol, said second solution comprises about 2-4 vol % glycerol, and said first solution comprises about 4-6 vol % glycerol.

9. (currently amended) The ~~process method for establishing undifferentiated human embryonic stem cells~~ of claim 5, wherein at least one of said treating steps is carried out for about 4-6 minutes.

10. (currently amended) The ~~process method for establishing undifferentiated human embryonic stem cells~~ of claim 5, wherein said first solution and said second solution each comprise about 15-25% human follicular fluid.

11-12. (canceled)

13. (currently amended) An undifferentiated human embryonic stem cell culture formed using the ~~method of any one of claims 1-12 process of any one of the preceding claims~~.

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14. (currently amended) A process for making method for establishing undifferentiated human embryonic stem cells comprising the steps of:

- (a) obtaining at least two cryogenically stored human embryos, wherein said at least two embryos consist solely of embryos in the blastocyst phase;
- (b) thawing one or more of said at least two embryos; and
- (c) removing trophectoderm from said embryos using anti-human lymphocyte antibody to isolate inner cell mass; and
- (d) culturing at least a portion of each of said ~~one or more thawed embryos~~ inner cell mass on a medium capable of sustaining undifferentiated embryonic stem cells; whereby undifferentiated human embryonic stem cells are established.

15-31 (canceled)

32. (new) The process of claim 1, wherein the culturing step is carried out in the presence of mouse embryonic fibroblast STO cells.

33. (new) The process of claim 14, wherein the culturing step is carried out in the presence of mouse embryonic fibroblast STO cells.